5

Patent P07 41795

ABSTRACT OF THE DISCLOSURE

Disclosed is a method of obtaining selectable transgenic stem cells of a vertebrate by transfecting a male germ cell with a transfection mixture comprising a nucleic acid construct containing a transcriptional unit of a stem cell-specific promoter, for example, a cyclin A1 promoter, operatively linked to a gene encoding a fluorescent or light-emitting reporter protein. The transfection mixture is a composition for transfection, in vivo or ex vivo, of a vertebrate's male germ cells, which comprises a nucleic acid or transgene, and a gene delivery system, and optionally a protective internalizing agent, such as an endosomal lytic agent, a virus or a viral component, which is internalized by cells along with the transgene and which enhances gene transfer through the cytoplasm to the nucleus of the male germ cell. In stem cells, other than germ cells, grown in vivo, expression of the reporter gene from a cyclin A1 promoter is facilitated by preventing methylation of promoter DNA by the use of flanking insulator elements in the nucleic acid construct. Alternatively, inhibitors of DNA methylation can be used in an in vitro growth medium. A kit contains components of the transfection mixture. Selectable transgenic stem cells have stably integrated the DNA, and non-human transgenic vertebrates comprise these selectable transgenic stem cells.

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